FURTHER OBSERVATIONS ON THE TRANSMISSION OF CUTANEOUS LEISHMANIASIS TO MAN FROM *PHLEBOTOMUS PAPATASII*

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PLATES XVI-XVIII

From October to December, 1924, and from April to December, 1925, sandflies were systematically collected in Jericho, with the object of determining the infection rate of sandflies with *Herpetomonas* in an endemic centre of cutaneous Leishmaniasis.

In 1924 three female *P. papatasii* were found positive (out of a total of two hundred and twenty, of which one hundred and seventy-four were females) and it was expected that systematic collection and dissection of a large number of sandflies during 1925 would yield ample material for a study of *Herpetomonas papatasii*.

During 1925, three thousand eight hundred and fifty sandflies from Jericho were dissected. Of these

132 were *P. papatasii* ♂♂
3,624 were *P. papatasii* ♀♀
13 were minutus group ♂♂
81 were minutus group ♀♀.

Three distinct species are included in the minutus group, *P. minutus*, *P. africanus* (syn. *P. minutus* var. *africanus* Newstead, 1912) and one undetermined species. A number of males of *P. papatasii* and of the minutus group were mounted and are not included in the above figures.

Of the total number of sandflies from Jericho only four (*P. papatasii* ♀♀) were found to contain *Herpetomonas*, i.e., an infection rate of, roughly, one per thousand. The result is rather

disappointing, particularly as Wenyon (1912), working in Aleppo, found 6 per cent. of sandflies infected with *Herpetomonas*. (During 1925 only four cases of cutaneous Leishmaniasis, diagnosed first clinically and then microscopically, were found in Jericho.)

The sandflies containing *Herpetomonas* were all captured in houses where there were no clinical cases of cutaneous Leishmaniasis, but it cannot be inferred from this (in spite of the fact that sandflies seldom if ever pass from house to house), that the source of infection in the sandflies was non-human, for, as we shall show later, an insignificant papule, clinically not recognisable as cutaneous Leishmaniasis, may nevertheless contain Leishman-Donovan bodies. Such papules would be overlooked, particularly in localities where insect bites are common.

In addition to the sandflies from Jericho, two hundred and fifty sandflies from Jerusalem, one hundred and eighty from Mozza, and one hundred and twenty from Haifa (all *P. papatasii* females) were dissected, and all were found negative for *Herpetomonas*.

The sandflies were collected in Jericho by one individual and about five hours were spent on each occasion in collecting from as many houses as possible, so that each catch was fairly representative of the *Phlebotomus* population of Jericho except that, as far as possible, females were caught. On April 20th, 1925, one hundred and seven sandflies were captured, and from April 30th to November 17th, 1925, an average catch amounted to about two hundred sandflies, the number varying from one hundred and seventy to two hundred and forty-six. Between November 17th and November 27th, sandflies diminished in numbers rapidly in Jericho, for on the former date two hundred and nine were captured, and on the latter date only one hundred and nine were caught. On the 7th December, 1925, only twenty-two were caught, and these were not found free in rooms, but were poked out of cracks in walls.

THE MORPHOLOGY OF *HERPETOMONAS* IN NATURALLY INFECTED SANDFLIES

Of the total of seven positive sandflies found in Jericho during 1924 and 1925, four contained mammalian blood and three contained no blood at all. Material from each of the latter was used for inoculation experiments on man.

The morphology of the *Herpetomonas* was studied in the fresh and in alcohol-fixed preparations stained with Giemsa.

The parasites show an extraordinary degree of polymorphism which renders their classification difficult. They may be divided into two distinct groups, one being a-flagellar and the other possessing a flagellum.

FORMS WITHOUT A FLAGELLUM

1. Leishmaniform bodies with one or two nuclei and one or two blepharoplasts. These bodies were found both in the stomach and, in one instance, in a smear of the upper part of the cardia (Pl. XVI,figs. 1-11).

2. Leishmaniform bodies, 3μ by 4μ, containing a single nucleus, a blepharoplast and a rhizoplast. These bodies were found in one instance in a smear of the upper part of the cardia containing the oesophageal valve (Pl. XVI, figs. 12 and 13).

3. Large round or oval parasites, 5μ to 12μ by 5μ to 8·5μ (Pl. XVI, figs. 14-25), with a varying number of nuclei and blepharoplasts. The evolution of this type appears to be as follows ; a large round body is found containing a central nucleus and one blepharoplast (Pl. XVI, fig. 14) ; the blepharoplast divides, and then division of the nucleus follows ; in some instances the blepharoplast divides several times before nuclear division commences ; finally a protoplasmic mass containing a varying number of nuclei and blepharoplasts is formed ; these masses must be considered as schizogony forms, for ultimately the protoplasm divides and a number of merozoites, flagellated and non-flagellated, are produced ; in some cases the flagellae of the daughter flagellates appear before division of the protoplasm is complete. Similar forms are found in cultures of *H. tropica* a day after the addition of specific immune serum to the culture.

The above form was found in a smear of the upper end of the cardia.

None of the above a-flagellar forms which were observed possessed a cyst wall ; they were all found together in a smear of the upper end of the cardia of a sandfly which was caught on the 26th of October, 1925, and dissected on the 2nd of November, 1925. Between these

two dates the sandfly had no opportunity of feeding. Forms No. 1 were also found in the stomach of sandflies which contained no parasites in the cardia ; these facts, taken in conjunction with the results obtained from artificially infected sandflies, lead to the conclusion that form No. 3 is a late stage in the development of the *Herpetomonas* in the sandfly.

FORMS WITH A FLAGELLUM

The classification of these forms must be artificial, for the various types tend to merge one into the other and there is no sharp dividing line between them. The following types may, however, be distinguished :—

1. Short, very thin forms (body 6·3μ to 8·5μ by 0·4μ to 0·8μ), with a flagellum up to 21μ long (Pl. XVI, figs. 40-45).

2. Long, very thin forms in which the body is scarcely thicker than the flagellum. Body 12·5μ to 17μ by 0·4μ to 0·8μ. Flagellum up to 21μ long (Pl. XVI, figs. 46-48).

These forms were observed in the stomach. They move very rapidly across the field, and, owing to the fact that their bodies are flexible and scarcely thicker than the flagellum, they present, on examination in fresh preparations, a superficial resemblance to long, thick spirochaetes.

3. Small, round or pyriform bodies with a short flagellum (body 3μ to 3·3μ by 1·7μ to 2·5μ) (Pl. XVI, figs. 26-28). These were found in a smear of the upper part of the cardia.

4. Large, round or spindle-shaped bodies (5μ to 13μ by 3·3μ to 8μ), with a flagellum up to 24μ in length (Pl. XVI, figs. 29-38). These forms were observed throughout the whole of the midgut.

5. Irregular forms filled with numerous vacuoles and chromatic granules (Pl. XVI, fig. 39). Although these appear to be degenerating forms they are, nevertheless, capable of active division.

6. Long thick flagellates (body 20μ to 23μ by 2·5μ to 3μ), with a flagellum up to 30μ long. These forms are specially numerous in the cardia. They were found in one instance in the proboscis, lying coiled up in chains along the upper surface of one mandible (Pl. XVI, figs. 70 and 71).

7. Intermediate forms (body 8·5μ to 15μ by 1μ to 3μ), with a flagellum up to 18μ (Pl. XVI, figs. 53-64).

The position of the nucleus in the flagellar types is very variable ; usually it is central or almost central, but it may be anterior (Pl. XVI, fig. 46) or posterior (Pl. XVI, fig. 59).

Division of the flagellar types of the *Herpetomonas* from sandflies takes place as follows ; the blepharoplast divides and this division is followed by the formation of a new additional flagellum usually much shorter than the old flagellum ; subsequently the nucleus divides, and then the body divides longitudinally from before, backwards along two fine, closely-set lines which appear to run throughout the whole length of the body.

8. Multiple division forms were found in one sandfly in a smear of the upper part of the cardia. In these forms the blepharoplast and nucleus divide a number of times, the original flagellum is retained or may become rudimentary, and ultimately division of the protoplasm takes place and a number of flagellates are produced (Pl. XVI, figs. 72-75). (Such forms are also found in old cultures on Noguchi medium or its modifications according to Wenyon or Kligler.)

Clusters of flagellates (Pl. XVI, fig. 76) were found in the cardia. Numbers of all types of *Herpetomonas*, flagellated and non-flagellated, were found to contain vacuoles and chromatic granules in their protoplasm.

DISTRIBUTION OF THE *HERPETOMONAS* IN THE ALIMENTARY TRACT OF *P. PAPATASII*

In four of the positive sandflies (those containing mammalian blood) the majority of the *Herpetomonas* were found mainly in the stomach. In the other three they were found in the oesophagus, oesophageal diverticulum, midgut and hindgut. The majority of the flagellates occurred in the cardia, particularly in the upper part of it ; they were found attached in large numbers by their flagella to the oesophageal valve and the epithelium of the cardia. In one of the sandflies they were also found in the pharynx, buccal cavity and the proboscis.

EXPERIMENTS ON THE TRANSMISSION OF CUTANEOUS LEISHMANIASIS TO MAN

Experiment No. 1 (previously recorded)

P. papatasii ♀, caught in Jericho, 25.6.25. Dissected 26.6.25. The whole alimentary tract behind the pharynx contained flagellates, the majority of which were long and rather thick forms (flagellar forms type 6). They were particularly numerous in the upper part of the cardia, where large numbers were attached to the posterior surface of the oesophageal valve.

Material was inoculated into two scarified points on the left forearm of a volunteer.

Result. 31.7.25. A small papule was noted on one inoculated point and on examination was found to contain Leishman-Donovan bodies. Early in December, 1925, the papule commenced to scale. Seven and a half months after the inoculation the papule was 2·0 mm. in diameter. Dr. H. Dostrowsky, dermatologist to the Rothschild Hospital, examined the papule and stated that, clinically, it could not be diagnosed as oriental sore. Nevertheless, Leishman-Donovan bodies were still present (8.2.26), though cultures remained negative.

Experiment No. 2

P. papatasii ♀, caught in Jericho, 8.9.25. Dissected 9.9.25. *Herpetomonas* found in oesophagus and oesophageal diverticulum, midgut and hindgut. The parasites were swarming in the cardia, where large numbers were found attached to the epithelium of the cardia and to the oesophageal valve.

Types of parasites: Flagellated types 1, 4 and 7.

Material from this sandfly was inoculated into two scarified points on the left forearm of a volunteer. After two weeks all traces of the original inoculation had disappeared. The case was observed till 15th December, 1925, and the inoculated points were found negative for Leishman-Donovan bodies.

Experiment No. 3

P. papatasii ♀, caught 26.10.25, dissected 2.11.25. Between these two dates the insect had no opportunity of feeding. On dissection, *Herpetomonas* was found throughout the whole alimentary tract from the proboscis to the rectum. The upper part of the cardia was completely choked up by flagellates, large numbers being attached to the oesophageal valve and the epithelium of the cardia. The

oesophageal diverticulum contained large numbers of flagellates and was found contracting on an almost solid mass of flagellates immediately behind its junction with the oesophagus. The extent of the infection in this insect may be gauged by the fact that a smear made from the upper part of the cardia (including the oesophageal valve), which was dissected away from the remainder of the midgut after examination in the fresh, showed thousands of flagellates.

Every type of *Herpetomonas*, except the very thin, long forms, was found. This was the only instance where multiple division forms were found in a sandfly.

Material from this case was inoculated into two scarified points on the left forearm of a volunteer (Mr. M. Ber, of Jerusalem), to whom we tender our sincerest thanks.

27.11.25. The patient noticed two papules on the site of the inoculated points. His attention was drawn to them on account of itching. He was examined 29.11.25, and two papules, one about 0·7 mm. and the other about 4 mm. in diameter were noted (Pl. XVII, fig. 1). Smears from both showed numerous Leishman-Donovan bodies. Dr. Dostrowsky kindly examined the papules and stated that, clinically, the case (although suspicious) did not resemble a typical oriental sore. The papules gradually increased in diameter and scaling commenced. On the 17th of January, 1926, one papule was 1·8 cms. and the other 1 cm. in diameter. They were both covered with scales and the centre of each was covered by a scab (Pl. XVII, fig. 2). Dr. Dostrowsky again examined the patient and stated that the lesions differed from typical oriental sores in that the infiltration was not marked. Cultures were obtained on Kligler's modification of Noguchi's medium.

On February 8th the scales fell from the centre of each lesion ; and marked infiltration round the centre of the lesion was present. Dr. Dostrowsky examined the patient and stated that, clinically, they were typical oriental sores (Pl. XVII, fig. 3).

THE ARTIFICIAL INFECTION OF *P. PAPATASII* WITH *HERPETOMONAS TROPICA* FROM ORIENTAL SORES

The object of the following experiments was two-fold, viz., to determine if *H. tropica* is capable of developing in *P. papatasii* after a feed on an oriental sore, and to find by direct experiment whether *H. tropica*, in passing through the sandfly, remains infective to man, i.e., whether man himself is the reservoir of cutaneous Leishmaniasis.

The aetiology of oriental sore in the light of the *Phlebotomus* theory (which is proved) is still obscure because of the absence or rarity of locally acquired cases in localities where sandflies are plentiful and cases from endemic foci are constantly present. The authors (1925) supposed that a third factor, apart from sandflies and human cases, was necessary to explain the curious distribution of cutaneous Leishmaniasis in Palestine, and particularly its absence from Jerusalem, but recently Dr. A. Dostrowsky, of the Rothschild Hospital, found a case of cutaneous Leishmaniasis in a child of seven who had never left Jerusalem.

The Sergents, Lemaire G. and Senevet G. (1915), suspected lizards of being the reservoir of *Herpetomonas tropica*, and succeeded in obtaining cultures of *Herpetomonas* from the blood of the gecko (*Tarentola mauritanica*). Wenyon (1921) found *Herpetomonas* attached to the epithelium of the mucosa of the cloaca of *Chameleo vulgaris* in Egypt, and the authors found a specimen of *Acanthodactylus syriacus* from Binyaminah, near Haifa (a locality which is free from oriental sore), in which the small intestine contained numerous *Herpetomonas*, but no Leishman-Donovan bodies or *Herpetomonas* were found in the blood stream or in smears of the internal organs. The possibility of sandflies becoming infected with *Herpetomonas*, by feeding on lizards, or by the larva swallowing faeces of lizards, must be borne in mind, although the lizard theory also fails to explain the distribution of oriental sore.

It was decided, before dealing with lizards, to attempt to transmit cutaneous Leishmaniasis to man from sandflies infected by feeding on oriental sores.

Two cases were finally persuaded into submitting to feeding experiments with sandflies. One was a case of an ulcerated oriental sore acquired in Baghdad, and the other a non-ulcerating lesion acquired in Bethlehem. Both were particularly suitable for the purpose of the experiment, for simple blood smears made from the indurated margin of the lesions showed numerous parasites both free and intracellular. In such cases, on histological examination, parasites are found not only free and in the endothelial cells and leucocytes, but also in some of the endothelial cells lining the capillaries and small vessels of the affected area. In both cases parasites were so numerous that failure to infect any particular sandfly that fed on the lesion could not be due to the fact that the insect did not ingest Leishman-Donovan bodies with the feed.

The feeding experiments were conducted between 25.9.25 and 16.11.25, after which date both cases refused to submit to further experiments. Four hundred females, *Phlebotomus papatasii*, were given an opportunity of feeding on the lesions, and of these only one hundred and sixty-eight fed. The sandflies used for the experiments were not laboratory bred but were collected in Jerusalem, Mozzah (near Jerusalem), and Haifa, but in view of the fact that the infection rate of sandflies in Jericho, an endemic centre of cutaneous Leishmaniasis, was one in a thousand, and that the controls dissected from Jerusalem, Mozzah and Haifa were all negative, there is justification for considering that the infections subsequently noted in some of the sandflies were acquired as a result of the experimental feeds.

The sandflies used for the experiments were placed in specimen tubes three-quarters of an inch wide and two and a half inches deep, and three sandflies at a time were used for each feeding experiment ; (if more were placed in a tube they disturbed each other and refused to feed). Each batch of sandflies was allowed from ten to fifteen minutes for a feed ; it was found that if they refused to feed within fifteen minutes they refused to feed for the day. In several instances it was noted that an undisturbed sandfly interrupted its feed for a few minutes and then returned, made a fresh wound and completed its feed. The sandflies fed on the indurated skin not less readily than on normal skin. After feeding, the sandflies were transferred to test-tubes which were then closed with lightly-moistened cotton wool, and placed in a horizontal position (the wool must be moistened daily but no fluid must be allowed to enter the tube, for if the wings of *Phlebotomus papatasii* are even slightly moistened the insects adhere to the side of the tube and die). After the experimental feed the insects were allowed no further opportunities of feeding.

Of the total number of sandflies which fed on the lesions only sixteen were afterwards found to be infected with *Herpetomonas*, in spite of the fact that all or nearly all the insects must have ingested Leishman-Donovan bodies.

(Apart from the sandflies which fed on the two naturally infected cases of oriental sore, fourteen were fed on Case 1 of the experimentally transmitted cutaneous Leishmaniasis ; all fourteen proved to be negative on dissection a varying number of days after the feed, but owing to the small size of the lesion and the difficulty of manoeuvring the insect to feed on the actual minute site of the

lesion, the experiments were discontinued. By the time the second case of experimentally transmitted cutaneous Leishmaniasis appeared favourable for experiments no sandflies were available.)

The table on page 185 gives the data of each infected sandfly as observed during examination of freshly-dissected material.

The following experiments were performed immediately after the examination of fresh preparations in saline.

Experiment No. 4

18.10.25. The whole of the midgut and hindgut of sandfly No. 39 was placed into a small pocket of skin scratched into the left forearm of a volunteer. Owing to the fact that the midgut of *P. papatasii* female contains a powerful anti-coagulin, the wound was observed continuously for three-quarters of an hour, till a solid clot was formed, in order to make certain that the inoculated material was not carried away with the escaping blood.

Experiment No. 5

30.10.25. Material from sandfly No. 79 was inoculated into two scarified points on the left forearm of a volunteer. (The material for one inoculation was obtained by dissecting off the upper part of the cardia, including the oesophageal valve—this part contained about twenty flagellates. The other point was inoculated with saline containing flagellates removed with a fine capillary from the slide on which the dissection was made.)

Experiment No. 6

3.11.25. Material from sandfly No. 127 was inoculated into two scarified points on the left forearm of a volunteer.

Experiment No. 7

4.11.25. Material from sandfly No. 128 was inoculated into three scarified points on the left calf of a volunteer.

Experiment No. 8

5.11.25. Material from sandfly No. 154 was inoculated into one scarified point on the left forearm of a volunteer.

Number	Origin of sandfly	Origin of sore on which sandfly fed	Date of feed	Date of dissection	Remarks
24	Jerusalem	Baghdad	30.9.25	3.10.25	Sandfly found dead. Five flagellates found in the stomach, four thick spindle forms and one long spirochaetal form.
39	Jerusalem	Baghdad	11.10.25	18.10.25	Midgut swarming with flagellates. Many attached to the oesophageal valve and to the epithelium of the cardia. Flagellates also present in the hindgut. No flagellates in the oesophageal diverticulum or anterior to the midgut. The majority of the flagellates were of the long thick type.
50	Haifa	Baghdad	25.10.25	28.10.25	Flagellates in stomach. All dead.
58	Mozzah	Baghdad	25.10.25	28.10.25	Numerous flagellates in midgut, particularly in stomach. None attached to the valve or the epithelium of the cardia. Short thick flagellates found and some short and very thin flagellates. A few intermediate forms.
79	Mozzah	Baghdad	25.10.25	30.10.25	Flagellates in midgut, mostly in the cardia. Some attached to the valve and upper part of cardia. Most of the flagellates of the long thick type.
83	Mozzah	Baghdad	25.10.25	30.10.25	Thick, medium-sized flagellates in midgut. None attached to the valve or cardia.
86	Mozzah	Baghdad	26.10.25	30.10.25	Long thick forms attached to epithelium of cardia. Long spirochaetal forms in stomach.
127	Mozzah	Bethlehem	1.11.25	3.11.25	Stomach swarming with flagellates. Some flagellates in cardia but not attached to epithelium.
128	Mozzah	Bethlehem	1.11.25	3.11.25	Stomach swarming with flagellates. Flagellates also present in the cardia. None attached to valve or epithelium of cardia. Flagellates seen passing into oesophagus and from oesophagus into pharynx. None in the oesophageal diverticulum.
133	Mozzah	Bethlehem	2.11.25	4.11.25	Heavy infection in stomach and cardia. Flagellates found attached to epithelium of cardia and others in process of attachment, the flagellae boring into the epithelium.
140	Mozzah	Baghdad	1.11.25	4.11.25	Slight infection in cardia only. Some attached to valve and to epithelium of the cardia, others in process of attachment.
143	Mozzah	Bethlehem	2.11.25	5.11.25	Slight infection (twelve flagellates seen) with long, thick flagellates in upper part of cardia. A few parasites attached to valve, others to epithelium of cardia.
147	Mozzah	Bethlehem	1.11.25	5.11.25	A few long thick flagellates in the cardia.
154	Mozzah	Baghdad	30.10.25	5.11.25	Whole of midgut swarming with flagellates. Clumps of flagellates attached to valve and epithelium of cardia. Long, thick flagellates. Type 3 of the a-flagellar forms and Leishmaniform bodies found in a smear of the uppermost part of the cardia, including the valve.
159	Mozzah	Bethlehem	1.11.25	6.11.25	Flagellates on the whole of midgut in oesophagus, oesophageal diverticulum, hindgut. The upper part of cardia almost choked up with flagellates Many attached to valve and epithelium of cardia. Flagellates nearly all long, thick forms.
160	Mozzah	Bethlehem	1.11.25	6.11.25	Midgut swarming with flagellates, particularly cardia. Upper part of cardia almost completely choked up with parasites. Many attached to valve and epithelium of cardia. Flagellates also present in hindgut in oesophageal diverticulum, in oesophagus, pharynx, buccal cavity and proboscis. In proboscis flagellates are coiled up in chains. Some of them apparently dead. Type 3 of the a-flagellar forms found in a smear.

Experiment No. 9

6.11.25. Material from sandfly No. 159 was inoculated into one scarified point on the left forearm of a volunteer.

Experiment No. 10

6.11.25. Material from sandfly No. 160 was inoculated into one scarified point on the left forearm of a volunteer.

Thus a total of seven volunteers were used for the above experiments and a total of eleven inoculations were made; of these, five were made from flagellates which had developed two days in the sandflies, four from flagellates which had developed five days in sandflies, one from flagellates which had developed six days in a sandfly and one from flagellates which had developed seven days in a sandfly.

Material from the sandflies not used for transmission experiments was fixed in absolute alcohol and stained with Giemsa; the material left on the slides after the transmission experiments was also thus treated.

The feeding experiments recorded above are not sufficient to determine the whole cycle of development because none of the positive sandflies were examined before the second day after the feed, and thus the actual development of Leishman-Donovan bodies into *Herpetomonas* was not observed and no infection was found in sandflies dissected after the seventh day.

Examination of stained smears revealed the following facts.

1. Leishman-Donovan bodies, some of them showing stages of division, are found up to three days after a feed, i.e., at a time when there are already varieties among the flagellar forms. Some of the Leishman-Donovan bodies found were elongated and much bigger and thicker than some of the flagellar forms, and distinctly longer (length up to 10μ) than any parasites found in the original skin lesion. There is, therefore, evidence of differentiation even before exflagellation takes place.

2. Two days after a feed, very short, thin flagellates (type 1 of the natural infections), thick, spindle-shaped or round flagellates (type 4), intermediate forms (type 7), and the long, very thin

flagellates in which the body is scarcely thicker than the flagellum (type 2) are present.

3. The long, very thin forms are present up to and including the fourth day.

4. After the third day the long, thick forms (type 6) appear and tend to become the dominant type, but the other forms (except the very long, thin forms), particularly those previously referred to as intermediate forms, are present.

5. On the fifth day schizogony forms (a-flagellar) appear (found in Nos. 154 and 160, in No. 154 in a smear of the upper part of the cardia) and Leishmaniform bodies were found in one instance (in No. 154, in a smear from the upper part of the cardia). These forms were very scarce. Multiple division forms such as those described from one naturally infected sandfly were not observed. Combining the examinations of fresh and stained material we may draw the following conclusions.

The ingested parasites exflagellate in the stomach and there they develop and multiply by division during the first three days. Subsequently many of them pass into the cardia, where some attach themselves to the epithelium of the latter and to the oesophageal valve by their flagellae. After the third day development and multiplication take place mainly in the cardia, particularly in the upper part, which may be completely choked up by parasites.

Flagellates pass into the oesophagus, oesophageal diverticulum, pharynx, buccal cavity and proboscis in the canal formed, when the mouth parts are at rest, by the under surface of the epipharynx and the upper surface of one mandible.

Taking these facts into account, and bearing in mind that there is an interval between the commencement of biting and the entrance of blood into the food canal, there is strong evidence for the view that *Herpetomonas tropica* is introduced into the skin by the bite of *Phlebotomus papatasii*. It is true that some of the flagellates found coiled up in the proboscis were dead, and that the proboscis is not a suitable habitat for the flagellates, but since active division and development take place in the uppermost part of the midgut, from where flagellates may continually pass forward into the oesophagus and pharynx and thence to the buccal cavity and proboscis, the dead flagellates in the proboscis can be continually replaced by living ones.

The fact that *Phlebotomus papatasii* only very exceptionally passes fluid per rectum during the act of feeding further supports the above view. (*H. tropica* may further enter the skin by the crushing of an infected sandfly.)

The volunteers inoculated from the artificially infected sandflies were observed until 18.2.26, and the result in each case was negative. This surprising result may be due to the facts that :—

1. The observation period is not sufficiently long. This cannot account for all the negative results in view of the relatively short incubation periods in the two positive cases from naturally infected sandflies.

2. The parasite, in passing from man to the sandfly, becomes non-infective for man, i.e., man is not the reservoir of *Herpetomonas tropica*.

3. The end point of the biological development of *Herpetomonas tropica* in *Phlebotomus papatasii*, whereby infective forms are produced, was not reached. This view gains support from the fact that the variety of forms noted in one instance, of a naturally infected sandfly, material from which gave such striking results in Experiment No. 3, was not found in the artificially infected sandflies.

The authors are inclined to the last view, but a discussion would only be of theoretical interest at present, and the matter must be decided by further observations and experiments.

SUMMARY AND CONCLUSIONS

1. The infection rate of sandflies with *Herpetomonas* in Jericho was found to be about one per thousand during 1925.

2. The morphology of naturally occurring *Herpetomonas* in *Phlebotomus papatasii* is described.

3. Two out of three experiments in transmission of cutaneous Leishmaniasis from sandflies naturally infected with *Herpetomonas* were successful.

4. Sandflies were artificially infected with *Herpetomonas tropica* by feeding on oriental sores, 10 per cent. of the sandflies being infected.

5. Attempts to transmit oriental sore to seven volunteers, by eleven inoculations with material from sandflies artificially infected with *Herpetomonas tropica* were all negative within an observation period of three and a half to four months.

ADDENDUM

The volunteer used for Experiment No. 2 and examined on December 15th, 1925, with a negative result left Palestine for leave in Europe. He returned to Palestine on the 24th March, 1926, and was again examined. A hard nodule was found in the subcutaneous tissue beneath the site of one of the inoculated points. The nodule was not attached to the skin, which was perfectly normal, or to the deep tissues, and was freely movable. The patient first noted the nodule in January, 1926. The nodule grew rapidly in size, and by April 14th, 1926, was about 9 mm. long and 6 mm. broad, and still remained freely movable. The patient suffered no pain or inconvenience from the presence of the lesion.

On April 14th, 1926, the nodule was punctured and Leishman-Donovan bodies were found. (Cultures made on a modification of Noguchi's leptospira medium on 22.4.26 were examined 28.4.26 and found positive.)

The case was seen by Dr. A. Dostrowsky, dermatologist to the Rothschild Hospital, who stated that out of over a hundred cases of oriental sore examined by him in Palestine none were observed with such a lesion.

On April 20th, 1926, two more volunteers were each given two inoculations from puncture fluid from the lesion of the above-described case. In one case the inoculations were made intra-cutaneously, and in the other subcutaneously, in order to determine whether the lesion produced in Experiment No. 2 was specific or due to a deep inoculation of the flagellates.

The results will be recorded in a future communication.

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EXPLANATION OF PLATE XVI

Morphology of *Herpetomonas* in *Phlebotomus papatasii*.

Figs. 1 to 11. Leishmaniform bodies.
1 to 8, from a naturally infected sandfly.
9 to 11, from an artificially infected sandfly.
5 to 8, from a smear of the upper part of the cardia.

Figs. 12 to 13. Leishmaniform bodies with rhizoplasts. Natural infection. From a smear of upper part of cardia.

Figs. 14 to 25. Showing a-flagellar schizogony forms.
14, large round form with a single nucleus and blepharoplast.
15 to 17, a single nucleus and a number of blepharoplasts.
18 to 25, a varying number of nuclei and blepharoplasts.
19, 20, 21, 23, from artificially infected sandflies.

Figs. 26 to 28. Small pyriform parasites with a short flagellum.
26 and 28, from an artificially infected sandfly.

Figs. 29 to 38. Larger, round and spindle-shaped flagellates.
29, 31, 33, 34, from an artificially infected sandfly.
37 and 38, dividing.

Fig. 39. Irregular form with numerous vacuoles and granules in the protoplasm.

Figs. 40 to 45. Short, thin forms.
40, 42, 43, from artificially infected sandflies.

Figs. 46 to 48. Long, thin forms.
47 and 48, from artificially infected sandflies.

Figs. 49 to 52. Types of dividing forms.
49 to 51, from artificially infected sandflies.
52, appears to be an abnormal dividing form.

Figs. 53 to 64. Intermediate forms.
53, 54, 55, 56, 58, 59, 60 and 63, from artificially infected sandflies.

Figs. 65 to 71. Long, thick forms. All from artificially infected sandflies.
70 and 71 (semi-diagrammatic), from proboscis.

Figs. 72 to 75. Multiple division forms. From a smear of the upper part of the cardia. All from a naturally infected sandfly.

Fig. 76. Cluster of flagellates. Camera lucida drawings × 1200, except 70 and 71.


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EXPLANATION OF PLATE XVII

Development of cutaneous Leishmaniasis on upper part of forearm.

Experiment 3. (Inoculation from naturally infected sandfly, on 2.11.25.)

Fig. 1. Papules about two-thirds of natural size.

Fig. 2. Natural size, 17.1.25.

Fig. 3. Natural size, 11.2.26. (Slightly different view from 2.)